

Aconitase Assay Kit

(Catalog #K716-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Aconitase (aconitate hydratase; EC 4.2.1.3) is an iron-sulfur protein containing an $[Fe_4S_4]^{2+}$ cluster that catalyzes the stereospecific isomerization of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle, a non-redox-active process. Tissue contains two aconitases, a mitochondrial (m-) and a cytosolic (c-) aconitase. They are related, but distinctly different enzymes and are coded for on different chromosomes. Loss of aconitase activity in cells or other biological samples treated with pro-oxidants has been interpreted as a measure of oxidative damage. BioVision's Aconitase Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric assay for measuring Aconitase activity in biological samples. In the assay, citrate is converted by aconitase into isocitrate, which is further processed resulting in a product that converts a nearly colorless probe into an intensely colored form with a λ_{max} at 450nm.

II. Kit Contents:

Components	K716-100	Cap Code	Part No.
Assay Buffer	30 ml	WM	K716-100-1
Substrate	lyophilized	Blue	K716-100-2
Developer	lyophilized	Purple	K716-100-3
Enzyme Mix (200µl)	200 µl	Green	K716-100-4
Cysteine	lyophilized	Red	K716-100-5
$(NH_4)_2Fe(SO_4)_2$	lyophilized	Brown	K716-100-6
Isocitrate Standard (10µmol)	lyophilized	Yellow	K716-100-7

III. Storage and Handling:

Store the kit at -20°C, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Substrate: Dissolve with 220µl DiH₂O; sufficient for 100 assays.

Developer: Dissolve with 1.1ml Assay Buffer before use; sufficient for 100 assays.

Aconitase Activation Solution: Dissolve cysteine and $(NH_4)_2Fe(SO_4)_2$ with 0.5ml Assay Buffer separately, and store at -20°C. Take out 0.1ml cysteine and $(NH_4)_2Fe(SO_4)_2$ solutions and mix together to prepare fresh activation solution.

All the solutions except the activated Aconitase are stable for 1 month at -20°C. Ensure that the Assay Buffer is at room temperature before use. Keep samples, Enzyme Mix and Aconitase solution on ice during the assay.

V. Aconitase Activity Assay:

1. Sample Preparations:

Homogenize 20-40 mg tissue or 10^6 Cells on ice in 0.1 ml cold Assay Buffer; Centrifuge at 800 x g for 10 min at 4°C; Collect the supernatant for c-aconitase assay. For m-aconitase assay, centrifuge the supernatant at 20,000 x g for 15 min at 4°C and collect the pellet, dissolve into 0.1 ml cold Assay Buffer, sonicate for 20 seconds. Keep samples at -80°C for storage.

Add 10 µl activation solutions to 100 µl sample; incubate on ice for 1 hour to activate aconitase in the sample.

Add 2-50µl activated samples into each well, and adjust volume to 50µl. We suggest using a background control group as well as several doses of your sample to ensure the readings are within the linear range.

2. Isocitrate Standard Curve:

Dissolve into 0.5ml assay buffer to prepare 20mM isocitrate standard solution. Take 20µl 20mM standard solution and add 180µl assay buffer to prepare 2mM isocitrate standard solution. Add 0, 2, 4, 6, 8, 10µl 2mM Isocitrate Standard solution into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well Isocitrate standard. Bring the final volume to 50µl with Assay Buffer.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

Sample Reaction Mix

46 µl Assay Buffer
2 µl Enzyme Mix
2 µl Substrate

Background Mix

48 µl Assay Buffer
2 µl Enzyme Mix

Add 50 µl of the Sample Reaction Mix to each test samples, background control and Isocitrate standards. Mix well and incubate at 25°C for 30-60 min. Add 10µl Developer to each well, mix and incubate at 25°C for 10min. Measure at O.D.450nm.

4. Calculation:

Plot the Isocitrate standard curve. $\Delta OD = OD_{sample} - OD_{background}$, apply the ΔOD to the Isocitrate standard curve to get B nmol of isocitrate generated by aconitase in 30-60min.

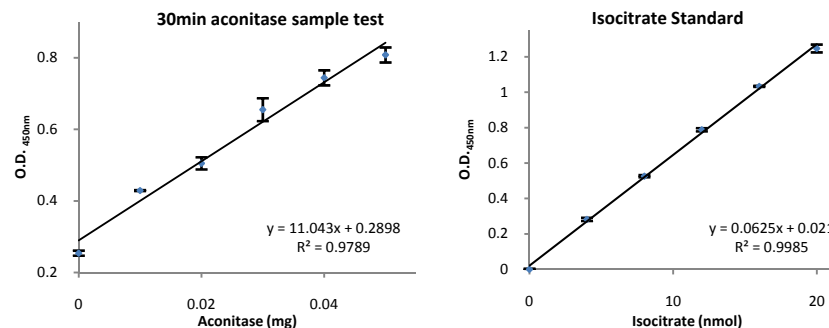
$$\text{Aconitase Activity} = \frac{B}{T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$$

Where: B is the isocitrate amount from standard Curve (in nmol).

T is the time incubated.

V is the pretreated sample volume added into the reaction well (in ml).

One unit will isomerize 1.0 µmol of Citrate to Isocitrate per min at pH 7.4 at 25 °C.



VI. Related Products:

Colorimetric Glutathione Detection Kit
Glutathione Kit (GSH, GSSG and Total)
GST Colorimetric Assay Kit
Acid Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
NAD/NADH Quantification Kit
Pyruvate Assay Kit

ApoGSH Glutathione Detection Kit
GST Fluorometric Assay Kit
Triglyceride Assay Kit
ADP/ATP Ratio Assay Kit
Phosphate Colorimetric Assay Kit
NADP/NADPH Quantitation Kit
Lactate Assay Kit/ II